100%), and 100% (range 56-100%) respectively. Cumulative incidence (CI) of grade II-IV acute GVDH while accounting for competing events, at day +100 was 34.2% (n=6) and 46.2% (n=8) at day +180. The CI for grade III-IV acute GVHD was 22% (n=4) at day +100, and 29.4% (n=5) at day +180. CI of chronic GVHD (cGVHD) was 50.5% at one year. CI of limited and extensive chronic GVHD was 22.1% (n=3) and 29.2% (n=4), respectively at one year. The CI of relapse was 11.7% (n=2) at one year. The non-relapse mortality (NRM) was 0% at day +100 and 14.7% (n=2) at one year. Causes of death include disease relapse (n=2), GVHD (n=2), and accident (n=1). The overall and progression free survival at one year is 73.5%. Our limited, retrospective data show encouraging relapse and NRM rates with TLI/ATG-based NMA conditioning, but with higher than previously reported rates of acute and chronic GVHD, underscoring the need for novel strategies designed to effectively prevent GVHD.

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Azithromycin Induces the Expansion of Regulatory T Cells and Diminishes Alloreactive T Cell Proliferation in Vitro and in Vivo

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Background: Donor T cell activation in response to alloantigens presented by host antigen presenting cells (APC) is a critical step in the pathophysiology of Graft Versus Host Disease. The macrolide Azithromycin (Azi) is studied in lung transplant and allogeneic hematopoietic cell transplant (HCT) recipients to decrease graft rejection and progression of lung injury, respectively. We now tested whether Azi plays a role in the modulation of adaptive immune responses by determining its effects on alloantigen-dependent T cell activation.
Methods: In vitro: Mixed lymphocyte reactions (MLR) using C57BL/6 (H2b) T cells as responders and B6D2F1 (H2bd) or BALB/c (H2d) splenocytes as stimulators, as well as TCR dependent, alloantigen-independent CD3/CD28 or lectin-type stimulation (Concavalin) assays of C57BL/6 Tcells were performed in the absence or presence of Azi at varying concentrations 5μg/ml, 10μg/ml, 20μg/ml, 50μg/ml. T cell proliferation was assessed by 3HT-uptake. T cells were phenotyped and supernatant was tested for cytokine expression.

In vivo: B6D2F1 were pretreated for 2 weeks with Azi at a dose of 3mg/ml drinking water or received untreated water as control. Then, animals were conditioned with 12Gy TBI and transplanted with 4X10E6 bone marrow cells and 6X10E6 splenocytes from allogeneic C57BL/6 donors. Recipients continued to receive Azi-supplemented or untreated water, and on day+7, splenic T cell expansion and T cell phenotyping were done.
Results: Alloreactive T cell proliferation in vitro showed an Azi dose-dependent decline at 96 hours along with reductions in TNF, IFNg, IL-2, IL-6, L-10, IL-17. Interestingly, a significant relative expansion of CD4+FOXP3+ T cells (Tregs) within the CD4+ T cell fraction was observed with increasing Azi-levels from 2.5±0.1% (0μg/ml) to 5.0±0.2% (50μg/ml). No differences in T cell proliferation were seen when T cell stimulation occurred APC-independent either via Concavalin A or via CD3/CD28 except for slight suppression at Azi 50 μg/ml. In vivo, when compared to controls, pretreatment with Azi resulted in decreased splenic expansion of both CD4+ T cells (2.1X10E6±0.2 vs. 4.3X10E6±0.6, $P < .01$) and CD8+ T cells (4.9X10E6±0.5 vs. 6.4X10E6±0.4, $P < .05$) along with a slight and non-significant increase of Tregs (1.5± 0.3% vs 1.1± 0.1%).
Conclusion: Azithromycin suppresses alloreactive T cell response in vitro and in vivo, paralleled by the induction of Tregs, supporting its use in lung transplantation and allogeneic HCT.

		Azithromycin (μg/ml)				
		0	5	10	20	50
MLR	cpm	20053 ± 1041	17497 ± 1237^{ns}	15340 ± 1157*	12902 ± 1317**	5637 ± 215**
	IFNg (pg/ml)	590.5 ± 59.9	476.0 ± 42.6	363.6 ± 18.6**	218.7 ± 19.2**	37.05 ± 4.5**
	TNF (pg/ml)	139.1 ± 8.0	114.2 ± 5.4*	115.4 ± 5.6*	104.6 ± 2.5**	71.3 ± 3.7**
	IL-2 (pg/ml)	27.0 ± 0.6	23.4 ± 1.2	23.4 ± 0.2	25.2 ± 1.0	18.3 ± 1.2**
	IL-10 (pg/ml)	115.5 ± 8.8	99.1 ± 14.4	98.9 ± 4.3	65.0 ± 6.8**	19.7 ± 3.5**
	IL-17 (pg/ml)	24.7 ± 0.9	19.3 ± 3.3	17.6 ± 1.1	9.4 ± 1.5**	4.2 ± 0.3**
	IL-4 (pg/ml)	—	—	—	—	—
	IL-6 (pg/ml)	169.3 ± 8.3	139.6 ± 16.3	120.7 ± 11.7*	96.7 ± 9.4**	49.9 ± 5.3**
	%CD4+FOXP3+	2.5 ± 0.1	2.9 ± 0.1	2.7 ± 0.3	4.2 ± 0.2**	5.0 ± 0.1**
Con A	cpm	61791 ± 4529	64350 ± 3692	66065 ± 3174		40012 ± 2016*
CD3/CD28	cpm	54868 ± 1873	51182 ± 2071	51351 ± 1447	51659 ± 862	42002 ± 633**

cpm = counts per minute
— = below assay sensitivity
* $p < 0.05$
** $P < .01$

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Association of Comorbidity Scoring with Outcome in Patients with Chronic Graft Versus Host Disease

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Background: Chronic graft versus host disease (cGVHD) is associated with mortality, disability and impaired quality of life. The importance of comorbidities in patients with cGVHD has not been reported, though this information could be important for prognostication and tailoring of treatment.

Patients and Methods: We studied 239 patients > 2 years of age (median 53y, range 11-79) diagnosed with cGVHD by the NIH consensus criteria and requiring systemic immunosuppressive therapy, enrolled in the cGVHD Consortium at the Fred Hutchinson Cancer Research Center (FHCRC). Both incident and prevalent cases were included. The Functional Comorbidity Index (FCI) and the Hematopoietic Cell Transplantation-specific Comorbidity Index (HCT-CI) were calculated for all patients at the time of transplant and cGVHD cohort enrollment. Global cGVHD severity by NIH criteria was mild or less in 8%, moderate in 54%, and severe in 38% of the patients. Median follow-up of survivors was 32 months (range 0.6-55.0). Univariate and multivariate Cox regression models were constructed, adjusting for all known and available risk factors including NIH global severity score at enrollment.

Results: The mean FCI score at the time of transplantation was 1.8 (range 0-8), increasing to 2.7 (range 0-7) at the time of cGVHD cohort enrollment ($P < .001$). The mean HCT-CI score at the time of transplantation was 2.6 (range 0-8), increasing to 3.7 (range 0-12) at the time of cohort enrollment ($P < .001$). Higher FCI scores did not predict risk of overall or non-relapse mortality. At enrollment, in multivariate analyses adjusted for clinical covariates, a statistically significant interaction of the HCT-CI with platelet count was observed ($p=0.003$). Higher HCT-CI scores at enrollment were associated with an increased risk of overall mortality when the platelet count was < 100,000/ml (HR 2.01 for 1-pt increase in HCT-CI: 1.20-3.35, $p=0.01$), but not when > 100,000/ml (HR 1.05: 0.90-1.22, $p=0.53$). For non-relapse mortality, higher HCT-CI scores at the time of cGVHD cohort enrollment were also predictive (HR 1.21:1.04-1.42,$P = .01$). Because of overlap between the HCT-CI and the NIH cGVHD severity scale in the scoring of liver (correlation=0.61) and lung (correlation=0.61) dysfunction, a version of the HCT-CI that excluded liver and lung variables was tested and remained significantly associated with overall survival in univariate analysis (HR 1.19,$P = .02$). Removing the NIH cGVHD global score from the model did not enhance the significance of the HCT-CI (HR 1.16,$P = .02$).

Conclusions: A higher comorbidity burden by the HCT-CI at the time of enrollment into a cGVHD cohort is associated with an increased risk for mortality. We recommend further studies of comorbidity scoring in patients with cGVHD and urge further investigations that could lead to a mechanistic understanding of physiologic vulnerability in patients with cGVHD.

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Interleukin-2 and Granulocyte-Macrophage Colony Stimulating Factor for Relapse After Allogeneic Stem Cell Transplantation

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Strategies for treatment of relapse after allogeneic stem cell transplant (SCT) include withdrawal of immunosuppressive therapy and donor lymphocyte infusions (DLI). Trials using cytokines (CK) have also demonstrated allo-immune responses after allogeneic SCT. We evaluated the role of interleukin-2 (IL-2) and granulocyte-macrophage colony stimulating factor (GM-CSF) on patients (pts) relapsing after allogeneic SCT.

Twenty three pts (median age=50, range 7 months to 66 years) received IL-2 at 1 million units/m2 subcutaneously (days 8-14) without (n=13) or with (n=10) GM-CSF at 500 mcg subcutaneously (days 1-14). Pts received a median of 2 cycles (range 1-4) of IL-2/GM-CSF. SCT sources included: peripheral blood matched-related (n=9), or unrelated (n=7), bone marrow matched related (n=1), or unrelated (n=2) or umbilical cord blood (n=4) SCT. Diagnosis included AML (n=12), MDS (n=3), ALL (n=2), non-Hodgkin's lymphoma (n=2) and Hodgkin's disease (n=1), Chronic Lymphocytic Leukemia (n=1). Disease status at SCT included CR1=3, CR2=1; all other pts underwent SCT for primary induction failure or resistant relapse. Median time from SCT to relapse was 5 months. Pts had received DLI (n=13) or chemotherapy (n=19) prior to CK therapy. Response rate after CK therapy was 57 % (13/23, all CR's). Median overall survival (see figure) after CK therapy was 9 months (range= 1-47 months). Acute or chronic graft versus host disease occurred in 13 pts after CK therapy. The table below summarizes analysis of immune activation. Values represent means +/- standard error at day 0 (first day of GM-CSF), day 8 (prior to start of IL-2) and day 14 (last day of IL-2 and GM-CSF). *P*-values are based on analysis of day 7 versus day 0 and day 14 versus day 0, respectively. Flow cytometric analysis showed an increase in the numbers of T-lymphocytes (CD3) and T-cell subsets (CD3/CD8 and CD3/CD4) as well as an increase in natural killer cells (CD16/56). Although no differences were seen in the number of dendritic cell subsets, DC1/DC2 ratios decreased with the administration of GM-CSF/IL-2. Limited (n= 4) CD4/FoxP3 analysis did not show change in absolute numbers with administration of GM-CSF/IL-2 (data not shown).

	D0 (Mean ± SE)	D7 (Mean ± SE)	D14 (Mean ± SE)	*P* value (Day 7-0)	*P* value (Day 14-0)
CD3 (k/uL)	309 ± 117	535 ± 103	1306 ± 403	0.034	0.027
CD3/CD8 (K/uL)	94 ± 42	174 ± 33	325 ± 90	0.021	0.029
CD3/CD4 (K/uL)	309 ± 117	404 ± 102	977 ± 310	0.249	0.045
CD16/CD56 (K/uL)	124 ± 60	404 ± 110	496 ± 162	0.029	0.044
CD19 (K/uL)	68 ± 39	89 ± 36	116 ± 31	0.183	0.044
Total Lymphs (K/uL)	488 ± 167	942 ± 160	2353 ± 532	0.016	0.013
CD11(DC1) (K/uL)	97.1 ± 60.7	46.3 ± 32.5	32.6 ± 27.5	0.108	0.101
CD123(DC2) (K/uL)	32.1 ± 7.3	39.7 ± 15.9	45.4 ± 35.8	0.694	0.628
DC1/DC2	2.77 ± 1.26	0.68 ± 0.35	0.61 ± 0.07		

In conclusion, CK therapy with IL-2 ± GM-CSF post SCT is associated with alloimmune responses. Relapse rate remains high with most pts relapsing after initial responses.